COMMENTARY

Setting molecular traps in yeast for identification of anticancer drug targets

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Almost 25 y have passed since Lee Hartwell et al. (1) proposed that systematic efforts to map genetic interactions in model systems promised to accelerate identification of new anticancer drug targets that exploit the unique molecular context of tumor cells. Synthetic lethal interactions, in which mutation of a gene causes cell death only when combined with mutation in another gene, are particularly relevant to cancer cells, given their many known genetic alterations (2). The promise of this concept has since been realized in the clinic: In fact, the synthetic lethality between poly(ADP ribose) polymerase (PARP) inhibition and recombination gene deficiency (e.g., *BRCA1* or *BRCA2*) has become a paradigm for personalized oncology (3) (Fig. 1*A*). Part of the effect of small-molecule PARP inhibitors is attributed to their ability to block the enzymatic activity of PARP while leaving DNA binding by PARP intact. The resulting "trapped" PARP–PARP inhibitor–DNA complex contributes to the antitumor activity of PARP inhibitors and so is thought to be a desirable property, although there are indications that trapping might also limit tolerability of PARP-inhibiting drugs (4). Nonetheless, small molecules capable of trapping their target on DNA are coveted due to the prospect of increased potency.

In PNAS, Hamza et al. (5) start with the premise that small molecules that cause trapping of protein–DNA or protein–protein complexes are desirable, and present a means of identifying trapping inhibitors by screening specific gene alleles in a yeast model (Fig. 1*B*). They propose that alleles with desirable properties (including DNA trapping) can show genetic interactions distinct from those shown by loss-of-function alleles, and that the resulting genetic interaction profiles can then be leveraged to identify small molecules with the same desirable properties (i.e., DNA trapping) as the initial allele (Fig. 1*C*). Recent and classic work by the yeast genetics community presents all the key aspects of a complete platform to generate and screen alleles of human disease-relevant genes to generate useful profiles for therapeutic small-molecule discovery.

Humanized Yeast

Yeast have long presented an outstanding tractable model to understand human biology and disease. Early examples of using yeast mutants to identify human genes, including *RAS* (6) and *CDC2* (7), have expanded to systematic studies to identify the complete set of human genes that can replace their yeast orthologs to create what are termed humanized yeast (8). The resulting humanized yeast can then be used in a myriad of assays to analyze the function of the human gene, most notably in screening small molecules for activity against human protein targets, including PARP inhibitors (9, 10). Interestingly, even in cases where an entire human gene does not complement deletion of its yeast ortholog, humanization of regions or specific amino acids of the yeast gene can be fruitful, particularly in modeling human disease-relevant mutations (11, 12). Also, if expression of a human gene elicits a growth phenotype in yeast, genetic screens to enhance or suppress the phenotype can reveal new disease-relevant biology [e.g., alpha-synuclein (13)]. The study by Hamza et al. (5) offers an interesting twist on humanized yeast. The authors show that a human gene, in this case *FEN1*, can be screened for genetic interactions in the presence of the normal yeast gene, or even in the presence of the normal human gene, identifying dominant synthetic lethal interactions (Fig. 1*B*). Thus, functional analysis of human genes in yeast models can be extended beyond human–yeast gene pairs that complement.

Building Mutant Alleles

Humanized yeast provides a simplified context for analyzing human gene function that is particularly amenable to systematic and high-throughput approaches. To make use of the humanized system, sources of

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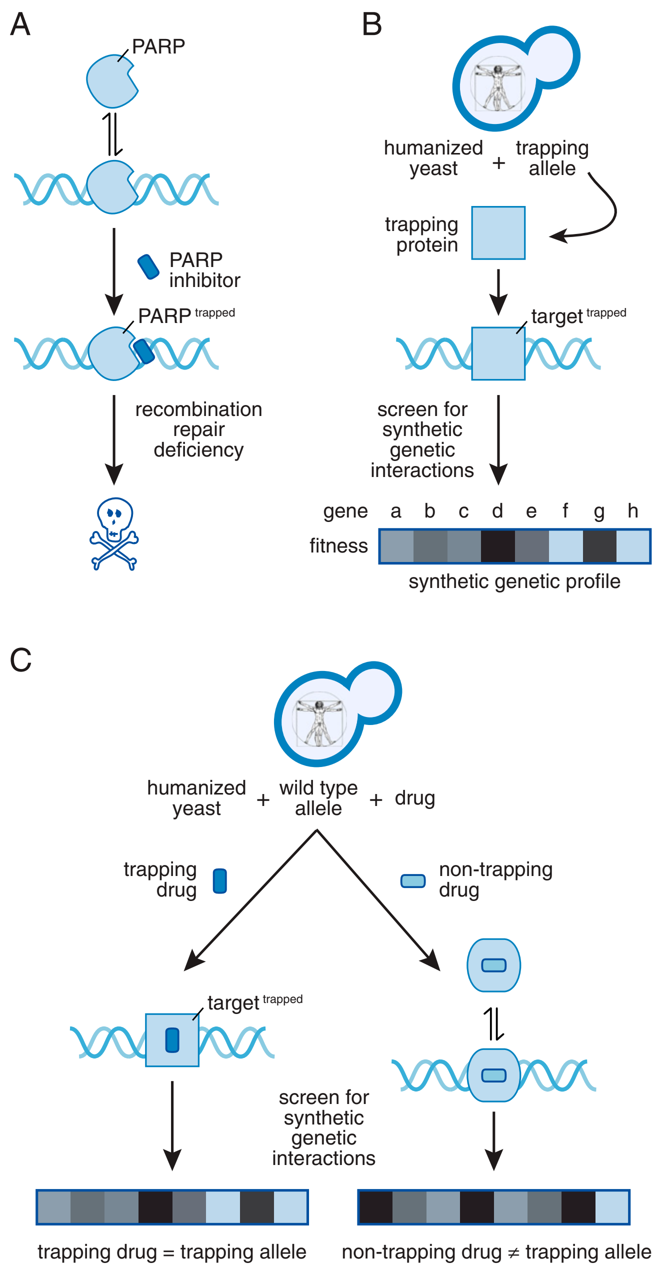

Fig. 1. **(*A*) PARP inhibitors can bind to and trap PARP on DNA, interrupting the normal PARP catalytic cycle. PARP inhibition and trapping is synthetic lethal with deficiencies in homologous recombination DNA repair genes. (*B*) Hamza et al. express an allele of human *FEN1* that encodes a putative trapping version of FEN1, in the presence of the normal *FEN1* gene. Systematic genetic interaction screening results in a genetic interaction profile for the trapping allele of *FEN1*. (*C*) Genetic interaction screening of small molecule inhibitors of FEN1 could present two possibilities. If the small molecule traps FEN1 on DNA, then the genetic interaction profile should strongly resemble that of the trapping allele (left branch). If the small molecule does not trap FEN1, then the profile should be distinct from that of the trapping allele (right branch).**

interesting alleles of human genes are necessary. Initial approaches focused on known alleles related to human diseases but have recently expanded to encompass "variants of unknown significance" and all possible amino acid substitutions. The deep mutational scanning technique pioneered by Fowler and Fields (14) allows the generation of single amino acid substitution alleles at the highest possible density. When connected to phenotypic assays, deep mutational scanning reveals mutant alleles with the desired properties, typically some kind of loss of function. Hamza et al. (5) screen *FEN1* alleles that were designed to inactivate catalysis but not DNA binding, and so were likely to result in trapping of FEN1–DNA complexes. Deep mutational scanning could be used to make separation of function mutants that lose catalytic activity but that retain protein–DNA or protein–protein interactions (15), exactly the type of trapping mutants that Hamza et al. model with their *FEN1* alleles.

Systematic Screening of Specialized Alleles

The effort of Hamza et al. (5) to discover genetic interactions associated with expression of a specific allele of *FEN1* complements and leverages major efforts in the yeast community to systematically map genetic interactions. The budding yeast is the only eukaryotic system for which a complete map of synthetic lethal interactions has been generated, through tests of all possible double mutants for synthetic growth defects (16, 17). Genetic networks can be mapped on this scale using methods for automated yeast genetics, which enable rapid generation of double mutants (17), and arrayed collections of yeast mutants carrying deletion alleles of nonessential genes or hypomorphic alleles of essential genes (for example, temperature-sensitive alleles) (18). Analysis of genetic interaction profiles, or the set of genes that show genetic interactions with a particular query strain, provides functionally rich information and identifies genes that share roles in various biological pathways and processes (17).

The genetic interaction profile associated with deletion of *RAD27*, the yeast version of *FEN1*, is highly correlated with those of other genes broadly involved in DNA replication and recombination and causes synthetic lethality with a large list of gene mutations (https://thecellmap.org/), including those identified by Hamza et al. as interacting with the trapping allele of *FEN1* (5). However, the dominant mutant allele of *FEN1* is only synthetic lethal with homologous recombination mutants, thus revealing a specific genetic interaction profile that a desirable trapping drug should match (Fig. 1*C*). Conceptually similar experiments have been performed using specific alleles of other genes involved in DNA replication/repair and genome stability, providing new insights. For example, the genetic interaction profile associated with a ubiquitination-deficient mutant of the replication clamp proliferating cell nuclear antigen (PCNA K164R) revealed a link to lagging strand replication that was not obvious in screens of other PCNA alleles (19). More broadly, comparison of the genetic interaction profiles associated with deletion of metabolic enzymes with those associated with the cognate catalytic mutants revealed moonlighting or noncatalytic functions for some enzymes (20). Remarkably, quantitative measurement of genetic interactions using panels of mutant alleles can also reveal structurally relevant functional information about proteins and protein complexes (21). For example, genetic interaction profiles associated with 350 mutations in yeast histones H3 and H4 provided orthogonal information to other data used for integrative modeling, reflective of the in vivo structure of histones (22). In general, comprehensive genetic interaction profiling of specific alleles is a promising avenue for discovery of new biology relevant to our understanding of the cellular mechanisms of human disease, and as Hamza et al. note, a promising avenue for drug discovery.

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Summary

Hamza et al. (5) provide proof-of-principle for an exciting approach to identify small molecules with therapeutic potential that act through specific mechanisms. Using a putative DNA–protein complex trapping allele of human *FEN1*, they combine a humanized yeast model with systematic genetic interaction screening to discover genetic interactions that should be recapitulated by a FEN1 inhibitor with trapping properties. In a manner analogous to the PARP inhibitor paradigm, Hamza et al. find that nuclease-dead alleles of *FEN1* are synthetic lethal with homologous recombination deficiencies. They predict that humanized yeast expressing *FEN1* in a recombination deficient background should be synthetic lethal with FEN1 inhibitors that cause trapping of FEN1–DNA complexes. The experimental platform can be readily extended to additional humanized yeast models of human disease genes and therapeutic targets, adding systematic generation of putative trapping alleles by deep mutational scanning. Panels of mutants can then be fed into specialized genetic interaction screens with increasingly sophisticated readouts, including fitness, morphology, protein abundance, and protein localization (23). The detailed genetic interaction profiles that result can form the basis for small molecule screens carefully honed to identify lead compounds with very specific properties.

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